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PRIMER NOTE

Isolation and characterization of 23 microsatellite loci in the hen flea *Ceratophyllus gallinae*

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Abstract

Nineteen dinucleotide, two trinucleotide and two tetranucleotide microsatellite loci developed for the hen flea *Ceratophyllus gallinae* are presented. Twenty fleas were screened at each locus. Loci were polymorphic (three to nine alleles per locus). These markers will provide a system to study population segregation and diversity, gene flow, dispersal and inbreeding.

Keywords: *Ceratophyllus*, flea, great tit, *Parus*, microsatellite, population

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Fleas are vectors for a range of economically important veterinary diseases. The study of vector biology requires an understanding of population level processes, such as migration, dispersal and inbreeding, and their effects on population dynamics and population genetics. Microsatellites have become one of the most important tools for studying these processes. In this report, we describe the isolation of 23 microsatellite DNA loci in *Ceratophyllus gallinae* and the development of appropriate polymerase chain reaction (PCR) primers.

Genomic DNA (50 μg) from a pool of 500 individuals was isolated using the Genomic Tip Kit from Qiagen (Basel, Switzerland). The DNA was sent to the Genetic Identification Services (Chatsworth, CA, USA; http://www.genetic-id-services.com) to develop four enriched microsatellite libraries containing inserts with $(CA)_n$, $(GA)_n$, $(TGA)_n$ and $(TAGA)_n$ repeats, respectively (Peacock *et al*. 2002). Cloning and sequencing procedures were as previously described (Binz *et al*. 2000). Primers for amplification of microsatellite loci were designed with the help of PRIMER3 (Rozen & Skatetzky 1996–97). Genomic DNA from 20 individual hen fleas originating from a single nest of a great tit pair was isolated using the DNAeasy Kit (Qiagen). The PCR reactions were carried out in a 10-μL volume containing 1 μL of the eluate containing the genomic DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.5 U of Ampli*Taq* Gold DNA Polymerase (Applera), 200 μM dNTPs, 0.5 μM of locus-specific fluorescent-labelled forward primer (fluorescent dyes were 6-FAM and HEX) and non-labelled reverse primer (see Table 1). The PCR cycling parameters were the following (in a Geneamp 2400 Thermocycler; Applied Biosystems): 10 min at 95 °C, 33 cycles at 95 °C for 30 s, 57 °C for 30 s and 72 °C for 75 s, followed by a final step at 72 °C for 7 min. Fluorescent PCR fragments were visualized by capillary electrophoresis on an ABI 3100 Genetic Analyser (Applied Biosystems).

All 23 loci analysed were polymorphic and their characteristics are summarized in Table 1. Heterozygosity values were calculated using the CERVUS software package (Marshall *et al*. 1998). For 11 loci, significant deviation from Hardy–Weinberg equilibrium (Table 1) could be detected using exact tests provided in the GENEPOP version 3.1d (Raymond & Rousset 1995). This may be due to a sampling effect since all fleas originated from the same nest where inbreeding may have occurred. For three loci (CgaCan5, CgaCAn19 and CgaGAn14), H_O was very low (0.06, 0.08 and 0.08, respectively), perhaps due to the presence of null alleles.

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Table 1 Primer sequences, repeat unit structure, expected allele size deduced from sequence, observed allele sizes, observed (H_O) and expected (H_E) heterozygosities and GenBank accession nos

Locus	Primer name	Sequence (5′–3′)	Repeat type	Expected allele size (bp)	Observed alleles (bp)	H_O	H_E	GenBank accession no.
CgaTGAn1	Cga2	F: CCACATATCTCTTCCGCTACG R: ATGTTCCGTCAGATGATTCG	$(TGA)_8$	201	189, 195, 198	0.4	0.45	AY159217
CgaTGAn10	Cga3As	F: CATTTTCCGATTCACTCAATTTC R: TGGAATAAACGATCCCCAAC	$(TGA)_8$	84	69, 72, 75, 78, 80	0.85	0.72	AY159218
CgaCAn21	Cga4s	F: CCTCGCATTACGAGAGTGC R: CGCGGTAAACCGATCCTAC	$(CA)_2(CG)(CA)_{19}$	150	138, 146, 148, 154, 156, 158	0.5	0.69	AY159219
CgaTAGAn2	Cga6	F: TTTTAACGGTGAATGTACCAATAC R: CAATACGGCGACATAACGTG	$(TAGA)_6$	173	165, 169, 171, 173, 175, 209, 217, 221	0.5*	0.81	AY159220
CgaGAn23	Cga9	F: ACGATCTGATGCCAAAAACC R: TTTCAACATGCATATGTTTACGTTAG	$(GA)_{12}$	181	165, 171, 173, 175, 177, 179, 181, 187, 193	0.6*	0.76	AY159221
CgaCAn22	Cga11	F: CGCCTACTGTGTGCCTAGC R: TCTGGAATCTTTATCATCCAACAC	$(CA)_{14}$	228	212, 224, 226, 228, 230, 232, 234, 236	0.38*	0.84	AY159222
CgaTAGAn7	Cga14	F: GCTAGATTGCTCGCTGGTAAG R: GTTTCATCTAATATCAATTTCCTCTCG	$(TAGA)_9$	302	246, 258, 282, 298, 302, 304, 306, 310	0.63	0.82	AY159223
CgaGAn26	Cga22	F: CGTCGTATTTGATATAACCAGACC R: TTGATCGTTTGATGATTTTCTG	$(GA)_{15}$	162	148, 152, 154, 158, 160 162, 164	0.3*	0.8	AY159224
CgaGAn29	Cga26	F: TTTCATAGAGGAATACCCTTTTCG R: TGTGTATCGTTTCGGAATGC	$(GA)_{15}$	215	203, 207, 211, 213, 215, 217, 221, 223, 225	0.74	0.82	AY159225
CgaCAn4	Cga28	F: CAATAAGGGGTCCGTGTGTC R: GGGGTGAAGGGTGTAATGTG	$(CA)_{16}$	277	263, 265, 267, 269, 273, 277, 279, 303	0.84	0.83	AY159226
CgaCAn5	Cga29	F: AATTGTGACTTCATGGCGAC R: GATGGTATTGTATTTATTGTTTGCG	$(CA)_{10}(AT)(CA)(TA)(CA)_8$ $(CTAACCTA)(CA)_7$	196	192, 210, 216, 230	0.06*	0.71	AY159227
CgaCAn6	Cga30	F: ATTCCCAACACACAGCGAC R: AACACGCGATACATTAATTTTG	$(CA)_{12}$	150	144, 150, 152, 160, 162, 166	0.75*	0.78	AY159228
CgaCAn7	Cga31	F: TATGCAAGATCACACCAGCC R: CACATTTCCCATCCCGTAAG	$(CA)_{16}$	216	198, 200, 202, 208, 216	0.7	0.65	AY159229
CgaCAn11	Cga32	F: GGTAGCTGGTGTGCGGTATC R: TGGATCAGGTGAGTCTGTGG	$(CA)_{11}$	135	127, 131, 133, 135	0.35*	0.73	AY159230
CgaCAn14	Cga33	F: TTCAACGTGTTTTTATCGCTG R: TAATTGCTCGCAATCCCTGG	$(CA)_{22}$	219	201, 203, 207, 213, 219, 221, 227, 257, 265	0.8	0.83	AY159231
CgaCAn17	Cga35	F: ACAAGCTGCGTCAGAAACAC R: TTTGTTATTTCGTCACCGCC	$(CA)_4(A)(CA)_{14}$	244	210, 218, 222, 252, 270, 272, 280	0.6*	0.79	AY159232
CgaCAn19	Cga36	F: ATTACACCCAGCACAGATGC R: GACTTCCTGTTTCATGTCGC	$(CA)_{12}(CG)(CA)_{13}$	230	180, 182, 184, 198	0.08*	0.63	AY159233
CgaGAn6	Cga40	F: TCTCCTACTCGGTTTTGTTTTG R: ATGGGGGAGGTTGTTTTATG	$(CA)_{13}$	121	117, 121, 123, 125, 127 129	0.38*	0.8	AY159234
CgaGAn9	Cga42	F: CTCGTCCCGTCATTCAGTTC R: GGATAGATGGATGGATAGCTGG	$(GA)_{10}$	156	142, 146, 150, 152, 154	0.9	0.77	AY159235
CgaGAn11	Cga44	F: CGAACATCAGTTTTCCAAAGG R: CCAGTAATAACACAACAATAACGC	$(GA)_{18}$	256	254, 256, 258, 264, 266	0.38	0.61	AY159236
CgaGAn12	Cga45	F: GCTATTCAGCGATAGGGGG R: GATCGTCGTAGCACATCACG	$(CA)_{13}$	159	155, 159, 163, 165, 167 169	0.88	0.7	AY159237
CgaGAn14	Cga46	F: TACTGCCTATCAAACGGACG R: TCCAAATGAAGTAGCGTTGC	$(CA)_8(CTAACCTA)(CA)_3$ $(TAC)(CA)_{11}$	172	172, 174, 176, 188, 190	0.08*	0.65	AY159238
CgaGAn16	Cga47	F: TGAGAGCTATGTGAGAAACAATAGAAG R: GTTCGTTGTGTGTGTGAGGC	$(GA)_{19}$	274	244, 248, 258, 260, 262, 264, 270, 272, 274	0.69	0.83	AY159239

*Significant deviation from Hardy–Weinberg equilibrium ($P < 0.05$).

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